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Neurokinin B Receptor Antagonism in Women with Polycystic Ovary Syndrome: A Randomized, Placebo-Controlled Trial

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ClinicalTrials.gov identifier: NCT01872078

Context: Polycystic ovary syndrome (PCOS), the most common endocrinopathy in women, is characterized by high levels of secretion of luteinizing hormone (LH) and testosterone. Currently, there is no treatment licensed specifically for PCOS.

Objective: To investigate whether a targeted therapy would decrease LH pulse frequency in women with PCOS, subsequently reducing serum LH and testosterone concentrations and thereby presenting a novel therapeutic approach to the management of PCOS.

Design: Double-blind, double-dummy, placebo-controlled, phase 2 trial.

Settings: University hospitals and private clinical research centres.

Participants: Women with PCOS aged 18–45 years.

Intervention: AZD4901 (a specific neurokinin-3 [NK3] receptor antagonist) at a dose of 20, 40, or 80 mg/day or matching placebo for 28 days.

Main outcome measure: Change from baseline in the area under the LH serum concentration–time curve over 8 hours (AUC) on day 7 relative to placebo.

Results: Of a total of 67 randomized patients, 65 were evaluable. On day 7, the following baseline-adjusted changes relative to placebo were observed in patients receiving AZD4901 80 mg/day: (1) a reduction of 52.0% (95% CI: 29.6–67.3%) in LH AUC; (2) a reduction of 28.7% (95% CI: 13.9–40.9%) in total testosterone concentration; and (3) a reduction of 3.55 LH pulses/8 hours (95% CI: 2.0–5.1) (all nominal $P < .05$).

Conclusions: The NK3 receptor antagonist AZD4901 specifically reduced LH pulse frequency and subsequently serum LH and testosterone concentrations, thus presenting NK3 receptor antagonism as a potential approach to treating the central neuroendocrine pathophysiology of PCOS.

Results: from this phase 2 clinical trial demonstrate the potential for a selective neurokinin-3 receptor antagonist to target the neuroendocrine pathophysiology of luteinizing hormone hypersecretion and hyperandrogenism in PCOS.

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women, and it affects approximately 5%–10% of women of reproductive age (1, 2). Different consensus groups have developed different definitions of PCOS. Depending on which definition is used, diagnosis is based on the presence of some or all of the following: chronic anovulation, polycystic appearance of the ovaries, and excessive testosterone secretion (hyperandrogenemia) or activity (hyperandrogenism) (3–5). PCOS is associated with several clinical presentations, such as menstrual dysfunction, infertility, hirsutism, acne, obesity, and metabolic syndrome (4, 6). In the long term, women with PCOS also have an increased risk of type 2 diabetes mellitus and potentially cardiovascular disease (6, 7). Treatment involves management of symptoms, chronic suppression of the hypothalamic–pituitary axis (eg, antiandrogens and exogenous sex steroids) or metabolic modulation (eg, metformin), often with therapies used off-label (6, 8). There is an unmet need to develop a targeted, safe, and effective treatment that addresses the underlying central endocrinopathy.

The pathophysiological mechanisms underpinning PCOS are multifactorial, including developmental factors, metabolic factors (eg, hyperinsulinemia) and genetic factors (9–11). Nevertheless, PCOS is associated with an increase in luteinizing hormone (LH) pulse amplitude and pulse frequency, which is likely driven by increased pulsatile secretion of gonadotropin-releasing hormone (GnRH) (12). This excess of pituitary LH secretion results in failure of ovulation and increased ovarian testosterone production (12). Recent discoveries suggest that the kisspeptin-neurokinin B (NKB)–GnRH pathway is the pivotal regulator of LH secretion (13, 14). Indeed, patients with genetically impaired NKB signaling have low baseline LH secretion and low LH pulse frequency (15, 16). Thus, pharmacological NKB blockade may be a useful approach to targeting the central pathophysiology of LH hypersecretion and hyperandrogenism in PCOS.

In mammals, there are three tachykinin receptors, of which the neurokinin-3 (NK3) receptor appears to be associated with a reproductive regulatory role through its ligand NKB (17). AZD4901 is a high-affinity antagonist of the human NK3 receptor (18). It was initially developed for schizophrenia in 2007–2010 (as AZD2624) but did not meet its developmental efficacy goals for that indication (19). In common with other NK3 receptor antagonists, however, AZD2624 reduced LH and testosterone concentrations in healthy volunteers and patients without endocrine or reproductive disorders (18). At the time, a reproductive role for NKB was yet to be elucidated (15). Since then, much evidence has accrued that suggests that NKB has a central role in the generation of GnRH and thus

LH pulsatility (13). It is therefore thought that AZD4901 regulates pituitary LH and gonadal testosterone via modulation of GnRH pulsatility.

PCOS is a heterogeneous disorder, with multiple pathophysiological mechanisms (eg, insulin resistance) in addition to LH hypersecretion contributing to its development. In this randomized controlled trial (RCT), our intervention (AZD4901) specifically targets LH hypersecretion. We hypothesized that AZD4901 could reduce LH pulsatility and prevent LH and possibly testosterone hypersecretion in women with PCOS, and we investigated this hypothesis in a randomized, multicenter clinical trial.

Materials and Methods

Study design and participants

This randomized, double-blind, double-dummy, placebo-controlled, phase 2 trial (ClinicalTrials.gov identifier: NCT01872078) was conducted between June 2013 and October 2014. Patients were competitively recruited in nine centers in Germany, UK, and USA (Appendix 1). The study protocol was reviewed and approved by the Institutional Review Board and Ethics Committee governing each participating center, and the study was conducted in accordance with the Declaration of Helsinki.

Eligible patients were women aged 18–45 years with a body mass index (BMI) of 18–40 kg/m² and a clinical diagnosis of PCOS; it was also a requirement that any confounding diagnosis had been excluded by the investigator. Participants needed to meet all of the following criteria: (1) polycystic ovaries documented by ultrasound; (2) free testosterone > 85% of the upper limit of reference range (measured within 21 days prior to randomization at Arup Laboratories, USA. Reference range: 0.8–7.4 pg/mL for women aged 18–30 years, 1.3–9.2 pg/mL for women aged 31–40 years and 1.1–5.8 pg/mL for women aged 41–51 years); and (3) amenorrhea or oligomenorrhea (defined as ≤ 6 menses per year).

Women who were not permanently or surgically sterile were required to use effective nonhormonal methods of birth control, such as strict abstinence or use of effective nonhormonal methods of birth control by the participant or their partner for the duration of the study. Acceptable barrier methods of contraception included condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository.

Patients were excluded if they had total testosterone serum concentrations ≥ 5 nmol/L (as very high testosterone is often associated with alternative diagnoses such as androgen-secreting tumors), if they had serum follicle-stimulating hormone (FSH) concentrations > 10 IU/L (as a marker to exclude peri- or postmenopause) (20), or if they had menstruated within the last 30 days.

Women with uncontrolled hypertension/diabetes, or significant pulmonary, renal, hepatic, endocrine, or other systemic disease, or any other clinically relevant diseases or abnormalities as judged by the investigator were also excluded in this early phase clinical trial of an investigational medicinal product. In addition, pregnant women and those not using adequate nonhormonal

contraception were excluded. Full exclusion criteria are presented in Supplemental Table 1.

Randomization and masking

Participants were randomized equally to four treatment groups: AZD4901 20 mg once daily (20 mg/d), AZD4901 20 mg twice daily (40 mg/d), AZD4901 40 mg twice daily (80 mg/d), or placebo twice daily (Figure 1). These doses were selected based on data from previous dose escalation studies, including those in which ascending doses of AZD4901 up to 80 mg/d were administered to healthy volunteers. In these studies, significant LH and testosterone suppression was seen at 40 mg/d, allowing a range of safe doses to be included in this study to explore a dose-response relationship.

Sequential randomization was carried out in each study center by the investigator following a blinded computer-generated randomization scheme produced by Quintiles Early Clinical Development (London, UK) using the AstraZeneca Global Randomization system. To maintain study blinding, AZD4901 and/or matching placebo were administered such that two tablets were taken twice daily by all participants. The first dose of AZD4901 or placebo was administered on the morning of day 1. Participants were treated for 28 days.

Procedures and outcomes

The primary endpoint was the change from baseline in the area under the LH plasma concentration–time curve over 8 hours postdose (AUC) on day 7 relative to placebo. Day 7 was selected as the time point to evaluate LH because AZD4901 would have achieved steady state and because any confounding that may occur owing to a LH surge preceding spontaneous ovulation would be avoided. Women who had menstruated in the 30 days before the baseline visit (during which screening procedures and laboratory tests were undertaken) were excluded.

Secondary objectives were to evaluate: (1) the change from baseline in average total and free testosterone serum concentrations over 8 hours postdose (C_{avg}) on days 7 and 28 relative to

placebo; (2) the safety and tolerability of AZD4901; (3) the pharmacokinetics of AZD4901 and its major metabolite AZD12292232; and (4) the pharmacokinetic/pharmacodynamic effect of AZD4901 on LH and testosterone concentrations and on LH pulsatility parameters on days 7 and 28 relative to placebo.

Several exploratory endpoints were investigated: (1) the change from baseline in LH AUC on day 28 relative to placebo; (2) changes from baseline in FSH, estradiol, progesterone, prolactin, thyroid-stimulating hormone, and insulin-like growth factor-1 on days 7 and 28; (3) glycated hemoglobin concentration on day 28; (4) the impact of AZD4901 on health-related quality of life (HRQOL) from baseline to day 28; and (5) the impact of AZD4901 on PCOS-specific patient-reported outcomes as measured by changes from baseline on days 7, 14, 21, and 28.

In addition, two post hoc exploratory analyses were carried out to assess: (1) the absolute change from baseline in LH AUC: FSH AUC ratio at days 7 and 28 relative to placebo; and (2) the changes in LH AUC, total and free testosterone C_{avg} , and LH pulsatility parameters relative to placebo in patients with no biochemical evidence of ovulation (serum progesterone < 6 ng/dL [19.1 nmol/L] at all study visits). Patients with no biochemical evidence of ovulation are referred to as ‘nonovulating patients’ hereafter.

LH pulsatility assessments were carried out using peripheral venous blood samples collected at baseline and on days 7 and 28 at 10-minute intervals for 8 hours on these three days. FSH and total and free testosterone concentrations were assessed using samples collected at baseline and on days 7 and 28 before the morning dose and then every hour for 8 hours. Estradiol, progesterone, prolactin, thyroid-stimulating hormone, insulin-like growth factor, total and free thyroxine, and glycated hemoglobin were measured using single samples collected at baseline and on days 7 and 28. Analyses of total and free testosterone were performed using high-performance liquid chromatography (HPLC)

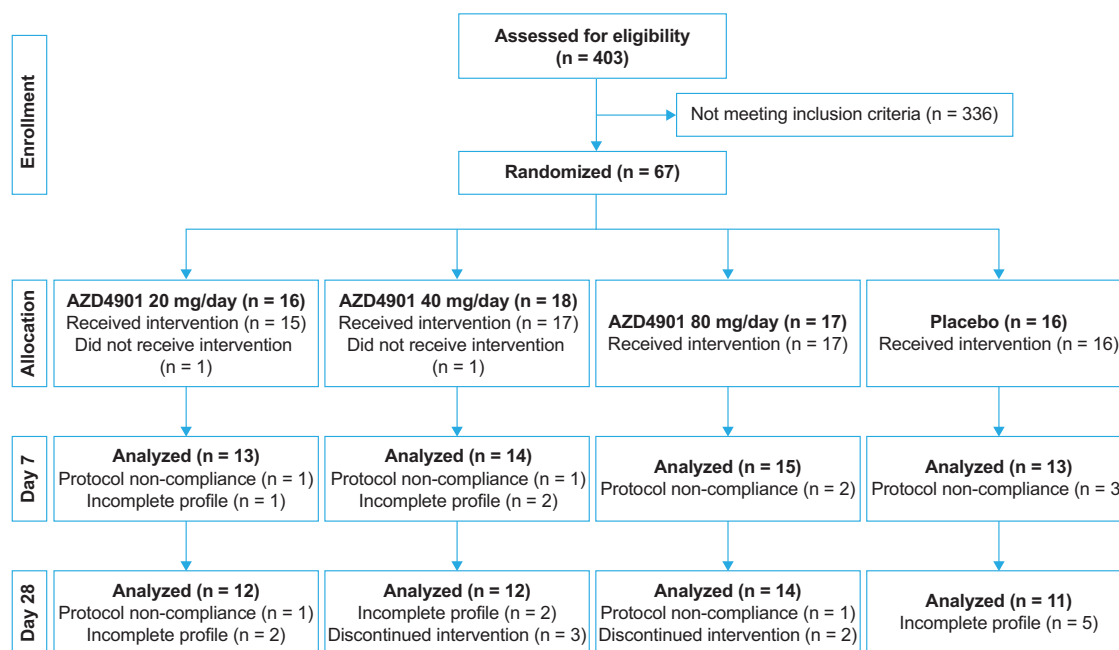


Figure 1.

tandem mass spectrometry. All other endocrine markers were analyzed immunometrically.

Samples for pharmacokinetic analysis were collected on days 7 and 28 before the morning dose and at 20 minutes, 40 minutes, and 1, 1.5, 2, 3, 4, 6, and 8 hours postdose. Health-related quality of life (QOL) was assessed using the 36-item Short-Form Health Survey (SF-36), completed by patients at baseline and on day 28. Safety assessments included adverse event monitoring, vital sign measurements, electrocardiograms, and physical examination. In addition, the Columbia-Suicide Severity Rating Scale (C-SSRS) was used to identify any suicide-related adverse events, including suicidal behavior and ideation, and was administered at baseline and each visit throughout the study. The C-SSRS was included because it had previously been mandated by the US Food and Drug Administration for the early clinical program of AZD4901 for the indication of schizophrenia.

Statistical methods

It was determined that a sample size of 48 patients (12 patients per treatment group) would be required to detect a 30% change from baseline in LH AUC on day 7 relative to placebo with 76% power at the two-sided 5% significance level. Allowing for potential drop-outs, it was planned to randomize 56 patients to achieve 12 evaluable patients per group. A total of 67 patients were actually randomized, 65 of whom were evaluable.

The dataset for analysis of pharmacodynamic parameters included all patients who received at least one dose of study medication (AZD4901 or placebo) and had appropriate pharmacodynamic measurement. The safety dataset comprised all patients who received at least one dose of study medication and for whom some postdose data were available. The pharmacokinetic analysis set comprised patients who received at least one dose of AZD4901 and had at least one postdose pharmacokinetic measurement without important protocol deviations or violations that could have affected the pharmacokinetic parameters significantly.

All analyses were performed using a mixed-effects model for repeated measures (MMRM) on change from baseline, with repeated-effects for day, fixed-effects for treatment, and treatment-by-day interaction. No adjustments were made for multiplicity.

LH AUC was calculated by linear up/linear down trapezoidal summation of observed serum concentrations. Data from day -1, 7, and 28 samples collected outside a ± 2 -minute collection window were not included in descriptive statistics for LH by time point. LH AUC was calculated if there were no more than five nonconsecutive missing values in the profile and no more than three consecutive missing values (no more than two consecutive missing values if one was at 0 or 8 hours). For LH AUC, comparisons between AZD4901 and placebo were performed using a MMRM on the ln-transformed ratio to baseline, with ln-transformed baseline LH AUC included as a covariate.

Data from day -1, 7, and 28 samples collected outside a ± 10 -minute collection window were not included in descriptive statistics for total and free testosterone serum concentrations by time. Total and free testosterone C_{avg} were calculated if there were no more than two consecutive or nonconsecutive missing values in the profile. If a value at 0 or 8 hours was missing, total testosterone C_{avg} was imputed using the next or previously scheduled value; free testosterone C_{avg} was not calculated if a value at 0 or 8 hours was missing. For total and free testosterone,

comparisons between AZD4901 and placebo were performed using a MMRM on the ln-transformed ratio to baseline, with ln-transformed baseline C_{avg} included as a covariate.

Pharmacokinetic parameters were derived using standard noncompartmental methods with WinNonlin Professional version 6.3 (Pharsight Corp., Mountain View, CA, USA) and descriptive statistics were reported. Descriptive statistics were also reported for HRQOL individual scale scores and for adverse events. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

Results from the MMRM analyses using ln-transformed data (eg, LH AUC) and nontransformed data (eg, number of pulses per 8 hours) were reported using geometric and arithmetic means, respectively.

LH pulsatility deconvolution analysis

The number of LH pulses, the mass-per-pulse (MPP), and LH basal secretion in 8 hours were derived using deconvolution analyses described previously (21, 22). Deconvolution estimates were calculated if there were no more than three nonconsecutive missing values in the profile and no more than two consecutive missing values in the profile. A MMRM on the ln-transformed ratio to baseline values was used for comparisons between AZD4901 and placebo for MPP and LH basal secretion, with the ln-transformed baseline values included as a covariate. For the number of pulses, comparisons between AZD4901 and placebo were carried out using a MMRM on the absolute change from baseline, with the baseline value included as a covariate.

Results

Study population

Of the 403 women assessed for eligibility, 67 met the inclusion criteria and were randomized to treatment; two patients did not receive an intervention because of difficult venous access, leaving 65 evaluable patients (Figure 1). The most common reason for noneligibility was failure to meet the screening criteria for free testosterone level (50% of screen fails); around one third of screen fails did not meet other laboratory inclusion parameters (most frequently, minor abnormalities in alanine transaminase and/or aspartate transaminase levels, iron-deficiency anemia and rare cases of elevated HbA_{1c}), while the remainder failed various clinical criteria including menstruation within the last month and BMI. Of the 65 evaluable patients, 15 patients received AZD4901 20 mg/d, 17 received AZD4901 40 mg/d, 17 received AZD4901 80 mg/d, and 16 received placebo. Demographic data and baseline characteristics for these 65 patients are shown in Table 1.

Primary endpoint

The baseline-adjusted changes of LH AUC at day 7 for the AZD4901 groups relative to placebo are presented in Figure 2A. In the AZD4901 80 mg/d group, there was a baseline-adjusted reduction in LH AUC of 52.0% (95%

Table 1. Patient demographics and baseline characteristics

	AZD4901 20 mg/day	AZD4901 40 mg/day	AZD4901 80 mg/day	Placebo
	(n = 15)	(n = 17)	(n = 17)	(n = 16)
Age, years ^a	29 (6)	27 (6)	28 (6)	27 (3)
Height, cm ^a	165.4 (6.2)	164.5 (8.1)	161.7 (4.5)	165.9 (7.4)
Weight, kg ^a	85.8 (16.9)	84.2 (17.0)	85.2 (18.6)	87.9 (20.2)
BMI, kg/m ^{2,a}	31.1 (5.9)	30.8 (5.6)	32.2 (6.2)	31.9 (6.6)
Race, n (%)				
White	15 (100.0)	13 (76.5)	11 (64.7)	14 (87.5)
Black or African American	0 (0.0)	3 (17.6)	3 (17.6)	1 (6.3)
Asian	0 (0.0)	0 (0.0)	2 (11.8)	0 (0.0)
Other	0 (0.0)	1 (5.9)	1 (5.9)	1 (6.3)
Ethnicity, n (%)				
Hispanic	2 (13.3)	2 (11.8)	1 (5.9)	3 (18.8)
Non-Hispanic	13 (86.7)	15 (88.2)	16 (94.1)	13 (81.3)
Serum hormone C _{avg} ^b				
LH, IU/liter	9.78 (3.49)	9.12 (4.59)	9.22 (3.76)	9.09 (5.09)
FSH, IU/liter	6.15 (2.06)	4.57 (1.67)	4.52 (1.49)	4.68 (1.35)
Total	1.96 (0.624)	2.07 (0.921)	2.25 (0.616)	1.68 (0.680)
testosterone, nmol/liter				
Free	66.3 (37.8)	72.3 (32.7)	91.9 (30.1)	84.5 (57.4)
testosterone, pmol/liter				

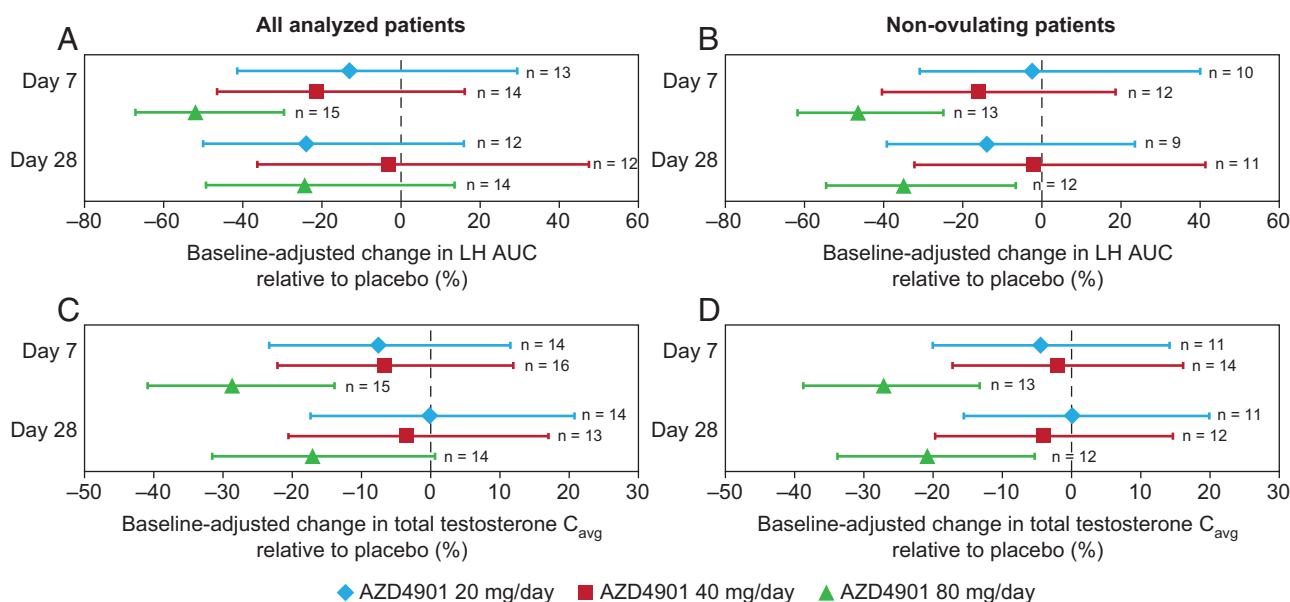
Data are arithmetic mean (standard deviation) unless otherwise stated.

^aAssessed during screening.

^bAssessed at baseline.

C_{avg}, average concentration over 8 h; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

CI: 29.6–67.3%; $P = .0003$) relative to placebo. There was no evidence of an effect on LH AUC change from baseline to day 7 relative to placebo for the lower AZD4901 doses.

**Figure 2.**

Secondary endpoints

Change in serum testosterone concentration

In the AZD4901 80 mg/d group, there was a baseline-adjusted reduction in total testosterone C_{avg} of 28.7% (95% CI: 13.9–40.9%; $P = .0006$) on day 7 relative to placebo. A corresponding reduction in free testosterone C_{avg} of 19.2% (95% CI: 0.14–34.62%; $P = .0486$) was also observed in this group on day 7. Similarly to LH, no significant reductions in testosterone concentrations from baseline to day 7 were observed in the groups receiving the lower AZD4901 doses relative to the group receiving placebo (Figure 2C and Supplemental Table 2). There was no evidence of an effect of any AZD4901 dose on total and free testosterone concentrations at day 28 (Figure 2C and Supplemental Table 2).

To explore the effect of AZD4901 on testosterone in women who were considered to have no biochemical evidence of ovulation in the study, a post hoc exploratory analysis that excluded patients with serum progesterone ≥ 6 ng/dL [19.1 nmol/L] (23) at any study visit was carried out. Nine women were considered to have ovulated during the study: three each in the AZD4901 20 mg/d and 80 mg/d groups, two in the AZD4901 40 mg/d group, and one in the placebo group. When these women were excluded, the baseline-adjusted reductions in total testos-

one C_{avg} from baseline to days 7 and 28 relative to placebo were 27.1% (95% CI: 13.3–38.7%) and 20.8% (95% CI: 5.3–33.8%), respectively, in the AZD4901 80 mg/d group (Figure 2D and Supplemental Table 2). Corresponding reductions in free testosterone C_{avg} were 22.8% (95% CI: 6.8–36.0%) and 23.8% (95% CI: 7.3–37.3%) (Supplemental Table 2). The baseline characteristics of nonovulating patients were not numerically different from that of the whole cohort.

Luteinizing hormone pulsatility parameters

LH pulse frequency and LH basal secretion were significantly reduced in the AZD4901 80 mg/d group on day 7 relative to placebo. There was a greater decrease in the number of LH pulses from baseline to day 7 in the AZD4901 80 mg/d group than in the placebo group, with the difference being 3.55 (95% CI: 2.0–5.1) pulses/8 hours (Figure 3A and Supplemental Table 2). The reduction in LH basal secretion from baseline to day 7 relative to placebo was 78.8% (95% CI: 53.6–90.3%) (Figure 3C and Supplemental Table 2); mass-per-pulse remained unchanged (Figure 3E and Supplemental Table 2). These effects persisted in nonovulating patients (Figure 3B and D and Supplemental Table 2).

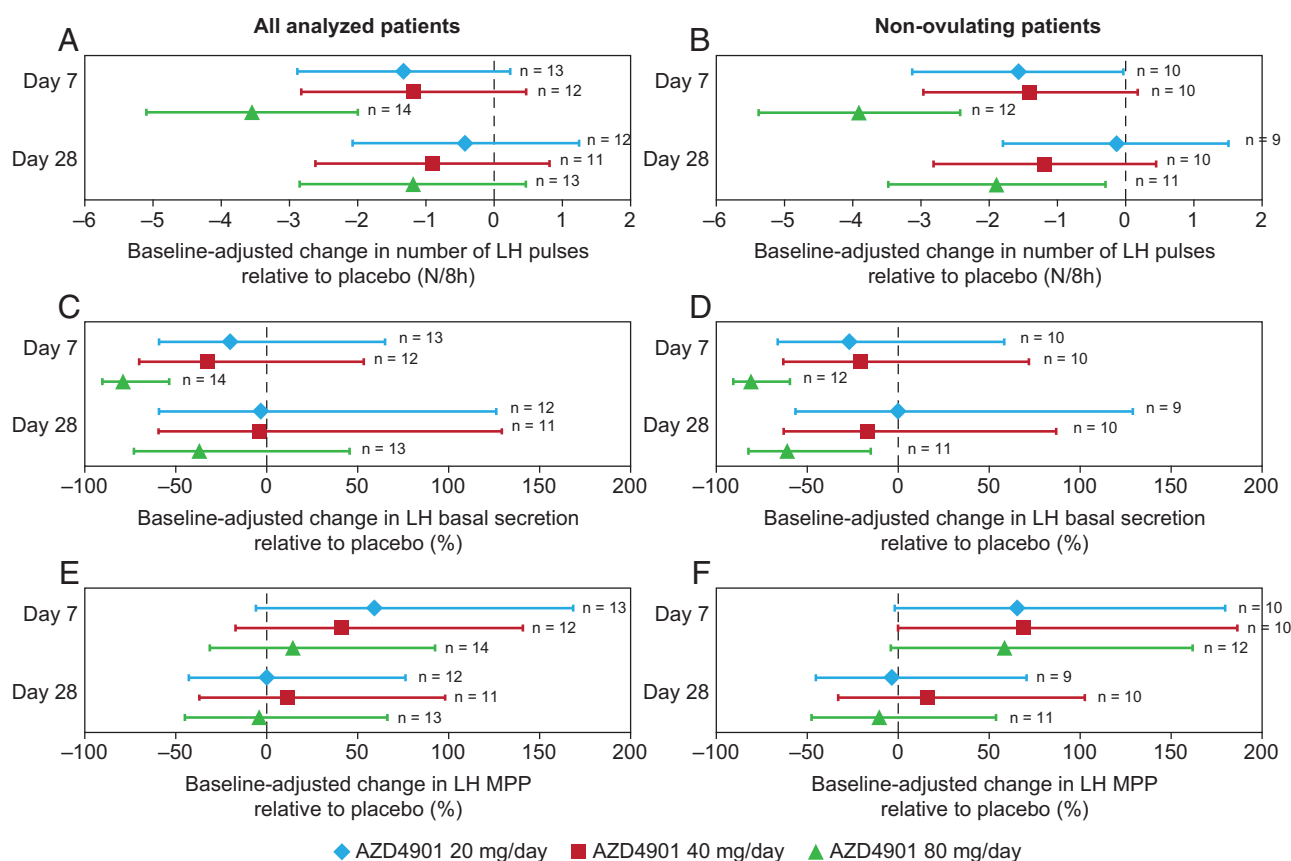


Figure 3.

Safety and tolerability

There was one serious adverse event reported in the study: a case of appendicitis considered by the investigator to be unrelated to treatment but which led to study discontinuation. This was the only adverse event that led to discontinuation. Overall, adverse events were reported by 32 out of 49 patients receiving AZD4901 (65.3%) and 8 out of 16 patients receiving placebo (50.0%). The most common preferred terms for adverse events reported by patients were headache (reported by 14 patients [21.5%]; six assessed by the investigators to be related to treatment), nasopharyngitis (reported by five patients [7.7%]; none assessed by the investigators to be related to treatment) and dizziness (reported by three patients [4.6%]; one assessed by the investigators to be related to treatment). No dose dependency was discernible in this small number of events (Supplemental Table 5).

No patients reported suicidal ideation or behavior on the C-SSRS questionnaire while receiving treatment or during follow-up.

Pharmacokinetic endpoints

Circulating concentrations of AZD4901 and its active metabolite increased in a dose-dependent manner that was consistent with the previously reported pharmacokinetic profile (19), and steady state was reached by day 7 (Supplemental Table 3). AZD4901 was quickly absorbed following oral dosing; time to maximum concentration was approximately 1.5–2 hours for all doses on days 7 and 28.

Exploratory endpoints

There was no evidence of an effect of AZD4901 on changes in LH AUC from baseline to day 28 relative to placebo (Figure 2A and Supplemental Table 2). In the post hoc analysis, when considering only nonovulating patients, the change in LH AUC from baseline to day 28 in the AZD4901 80 mg/d group was 34.9% (95% CI: 6.6–54.6%) relative to placebo (Figure 2B and Supplemental Table 2).

Relevant biochemical parameters are summarized in Supplemental Table 4. FSH concentrations remained largely unchanged in all treatment groups. Therefore, given the changes in LH AUC, there was evidence of an absolute reduction in the baseline-adjusted LH AUC:FSH AUC ratio relative to placebo. Reductions relative to placebo were observed on day 7 (0.70; 95% CI: 0.23–1.17) and day 28 (0.72; 95% CI: 0.23–1.21) in the AZD4901 80 mg/d group. There was no evidence that the lower AZD4901 doses had an effect on the LH AUC:FSH AUC ratio relative placebo (Supplemental Table 2).

There was no evidence of an effect of AZD4901 on HRQOL. Changes from baseline to day 28 across the

seven parameters of the SF-36 questionnaire were small across the four treatment groups, and there were no obvious trends (Supplemental Table 6).

Discussion

In this first study to manipulate the NKB–GnRH pathway in PCOS, the NK3 receptor antagonist AZD4901 specifically reduced LH pulse frequency and, subsequently, serum LH and testosterone concentrations. These reductions persisted in nonovulating patients until the end of the dosing period (day 28), although were not statistically significant in the whole group. The duration of dosing in this initial study was insufficient to assess the effect of AZD4901 on clinical outcomes; longer trials with greater patient numbers are needed to explore further the potential therapeutic role of AZD4901 in PCOS.

The present results are consistent with the hypothesis that modulation of the GnRH axis by NK3 antagonism using AZD4901 would decrease LH pulse frequency and lower LH and testosterone concentrations in women with PCOS. They are also consistent with previous data from early clinical investigations of NK3 antagonists in individuals without an endocrine or reproductive disorder. In these studies in healthy volunteers and patients with schizophrenia, dose-dependent decrease in LH and testosterone concentrations were observed (18). Taken together with a central role of NKB in the regulation of GnRH and LH pulse frequency, the present study demonstrates the potential of NKB antagonism to provide a novel approach to treating the central neuroendocrine pathophysiology of PCOS (ie, the LH hypersecretion that, in turn, drives androgen excess).

In this study, reduction in overall LH secretion was underpinned by reductions in LH pulsatility as well as in basal LH secretion but not in the amount of LH secreted per pulse. These findings are consistent with low LH pulsatility observed in patients with genetic defects leading to impaired NKB signaling (16). Therefore, the present study contributes to the recent insights obtained into the regulation of GnRH pulsatility following discoveries of hypothalamic roles for kisspeptin and neurokinin B (13, 24, 25).

The observed reductions in LH and testosterone concentrations from baseline seen with the highest dose of AZD4901 were statistically significant at day 7 for the study population, but not at day 28. Reductions were, however, statistically significant at day 28 in those women who did not ovulate during the study. This is because, given the small sample size, the day 28 LH and testosterone results were confounded by what appeared to be a

preovulatory LH surge in a small number of women; excluding women who had ovulated during the study from the analysis resulted in the maintenance of the reduction in LH and testosterone concentrations to day 28.

AZD4901 was well tolerated in patients with PCOS. Most adverse events were considered unrelated to the study medication by the investigators, including the single serious adverse event. Furthermore, given this class of compounds was initially developed for the treatment of schizophrenia, it is reassuring to note that questionnaires assessing participant well-being (SF-36 and C-SSRS) did not show any signals of concern.

Currently, while several medications are used to treat PCOS and its symptoms, there is no treatment with specific regulatory approval for PCOS, and there are very few new molecular entities in clinical development for this condition (8). Hence, a range of agents such as spironolactone, GnRH modulators, metformin, oral contraceptive (OC) pills and clomiphene are used to manage the symptoms and associated health complications of PCOS (6), reflecting the multifactorial etiology of the condition (9–11). The results from this study, if consistently reproduced in subsequent clinical studies, suggest that AZD4901 has the potential to emerge as a novel therapy for PCOS and to complement recent developments in the treatment of anovulatory infertility in women with PCOS (26).

The present study has clear strengths such as the inclusion of detailed LH pulse profiling and the use of placebo control to estimate placebo-adjusted changes from baseline hormones. Only the highest dose administered elicited a significant response in our study, suggesting that we may not have reached maximal response in this PCOS population and that testing doses higher than 80 mg/d in future studies may be warranted, a point supported by the safety and tolerability profile of AZD4901 in the present study. Before AZD4901 can be developed as a therapy for PCOS, longer studies assessing clinical outcomes (eg, ovulation and hirsutism) and quantification of potential metabolic improvements in larger populations, as well as potential compensatory mechanisms, are needed.

Because this was a phase 2a trial aimed at validating the concept, our focus was on biomarkers such as LH and testosterone; the duration of treatment was insufficient to assess the effects on clinical endpoints such as ovulation. A small number of patients appear to have ovulated during the trial, based on random serum progesterone > 6 ng/mL (19.1 nmol/L), which is consistent with clinical practice recommendations (23) and previous data (27). A total of nine women ovulated, three each in the 20 mg/d and 80 mg/d groups, two in the 40 mg/d group, and one in the placebo group. These small numbers did not allow us to

make any meaningful comparisons between groups, or between ovulators and nonovulators. While the observed ovulation rates suggested by elevation of serum progesterone concentration (particularly among treated patients) may be higher during our study than expected for PCOS patients in general, their possible relationship to treatment cannot be firmly concluded for at least two reasons: firstly, the small numbers of ovulating patients observed do not allow statistically rigorous comparisons among groups; secondly, the timing of ovulation within the study appeared to differ across the nine women but serum progesterone measurements were only taken at baseline and days 7, 28 and 42, so the day of ovulation cannot be precisely identified. Both reasons reflect the fact that this early study was designed and powered to achieve a different primary endpoint. Given the clinical importance of menstrual irregularity in PCOS, ovulation needs to be characterized further in future longer-term studies using self-reported menstruation (eg, menstrual diary), biomarkers (eg, LH, estradiol), and/or ultrasonography over multiple cycles.

The results of our study also have implications for wider research into new therapies for patients with PCOS. First, the heterogeneity of the PCOS phenotype presents a challenge to attaining adequate power in early-phase randomized controlled trials. We addressed this by selecting a hyperandrogenemic population with polycystic ovarian morphology and menstrual irregularity. Such an approach, however, required the screening of well over 400 women to recruit 67 participants. Furthermore, the generalizability of our results to nonhyperandrogenemic patients with PCOS requires further study.

Finally, it has to be emphasized that the present study is a clinical trial of a pharmacological agent; therefore, inferences on the etiology of PCOS and the multifactorial nature of the mechanism by which LH pulse frequency becomes increased cannot be drawn from the present data.

In conclusion, this is the first clinical study to manipulate the hypothalamic kisspeptin-NKB-GnRH pathway in women with PCOS. The NK3 receptor antagonist AZD4901 reduced serum LH pulse frequency and, subsequently, serum LH and testosterone concentrations. These findings demonstrate the potential for NKB antagonism to provide a novel therapeutic approach by targeting the neuroendocrine pathophysiology in PCOS.

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